b) mixing said sample with a PNA probe, said PNA probe having a sequence complementary to at least a portion of said target sequence,

c) adjusting the temperature of the mixture resulting from step b) from a temperature of about 95° - 65° C to a temperature of about 60° - 30° C;

d) separating a PNA probe/nucleic acid complex from other components of the mixture resulting from step c) to produce a separated PNA probe/nucleic acid complex when said target sequence is present; and

e) detecting said separated PNA probe/nucleic acid complex.

REMARKS

Claims 32-51, 53-62, and 64-67 are pending in this application.

Claims 32-37, 39-51, 53, 55, and 56 are rejected. Claims 38, 54, and 57 are objected. Claims 58 –62 and 64-67 are allowed.

Claims 32, 34-37, 46, 54, 55 and 58 are amended herein. Claim 53 is canceled without prejudice. Claims 68-74 are added.

Accordingly, claims 32-51, 54-62, and 64-74 are presented for examination.

Amendments to Claims

Without acquiescing with the Examiner's basis for rejection of the claims, claims 32, 34-37, 46, 54 and 55 have been amended to more particularly point out and distinctly claim the subject matter which Applicants regard as their invention. More specifically, claim 32 has been amended to recite that 1) the double stranded section of greater than 50 subunits is suspected to include the target sequence, and 2) the separating, not the mixing, "begin[s] in the presence of a medium denaturing to said double stranded section to produce a separated PNA probe/nucleic acid complex."

Claim 34 has been reworded to correct the antecedent basis of the claims, i.e., reference to the denaturing medium rather than denaturing reagent. Claim 35 has been amended to recite a low salt concentration of less than 50 mM. Support for this amendment can be found at least at

page 13, lines 3-11. Claim 36 has been amended to further recite a step of adjusting the temperature from about 95°–65° C to about 65°–30° C. Support for this amendment can be found at least at page 11, lines 9-12, at page 22, lines 12-13, and at page 24, line 15 to page 25, line 26. Applicants submit that no new matter has been added.

Claim 46 has been amended to recite with more particularity the detectable moieties and remove non-structural language. Objected to claim 54 was rewritten in independent form.

Claim 58 has been amended to correct a grammatical error.

New claims 68 to 70 are claims depending on allowed claim 58, and are directed to specific preferred embodiments. Support for these claims may be found at least in the drawings in Figs. 13 and 15, and in the specification at page 28, lines 9-15 and at page 29, lines 27 – 30. Applicants submit that no new matter has been added. New claim 71 has been added to claim a preferred embodiment of the microchip apparatus. Support can be found in the specification at least at page 27, line 25 to page 30, line 9, at page 30, line 29, to page 31, line 24, and in Figures 12, 13, and 15.

New claims 72-74 are directed to preferred embodiments of the methods of the invention. Support for PNA probe length of claim 72 may be found throughout the specification for example at page 12, lines 26-32, at page 19, line 17, at page 22, lines 8-9, and at page 24, table 2. Support for other limitations of claims 73-74 has been described *supra* for claims 34, 36, and 46. Applicants submit that no new matter has been added.

Rejection of Claims Under 35 USC § 103

Claims 32-37, 39-51, 53, 55 and 56 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Rose in view of Chen et al. and Nielsen et al. Applicants have amended the claims to more clearly define the subject matter which Applicants regard as their invention. Applicants clarified that the double-stranded section is suspected to include the target sequence. This amendment clarifies that the PNA probe competes with the complementary strands for binding with the target sequence of the nucleic acid. This competition was known to be strongly in favor of nucleic acid/nucleic acid formation, however, the denaturing conditions of the claimed invention favor the formation of a PNA/nucleic acid heterocomplex. Applicants

respectfully submit that the amendments to the claims obviate this rejection as neither Rose, Chen, or Nielsen, taken alone or in combination, teaches the instantly claimed invention as a whole.

As stated in the prior Amendment and Reply, Rose does not teach or suggest Applicants invention as is presently claimed in claims 32 and 46 and their depending claims. First, Rose only discloses short PNA sequences (12 units) binding to short oligonucleotides (12 units). Thus, Rose does not teach the formation of a complex between a PNA probe and a nucleic acid which is capable of forming a double-stranded section of at least 50 nucleotide subunits with its complementary strand as recited in claims 32, 46, and 72-75.

Second, Rose does not teach the formation of PNA/nucleic acid complexes in a medium denaturing to double stranded sections of nucleic acid of greater than 50 nucleotide subunits. Rose only describes mixing and separating procedures that are known to be non-denaturing for such long stretches of nucleic acids. Indeed, the double stranded oligonucleotide (ODN) is observed along with the PNA/ODN complex (see Fig. 7, page 3549, col. 1 page 3546, last line of the first paragraph following Experimental section). In those conditions, Rose shows that the only single strand present is the complementary strand that is displaced by the PNA, and in an amount equaling that of the PNA/ODN complex.

More specifically, Rose discloses denaturing conditions (heating from 30° to 60° C). However, teaching away from the presently claimed invention, these conditions also are denaturing to the PNA/ODN complex (see Fig. 3, page 3547). Thus, in reading Rose, one could not deduce the presently claimed invention in which a PNA/nucleic acid complex may be formed and separated from a medium that is denaturing to nucleic acid/nucleic acid complexes.

As stated in the prior Amendment and Reply, Chen does not cure the deficiencies of Rose. Chen merely teaches analysis procedure for nucleic acid/nucleic acid complexes. Chen only concerns hybridization between single-strand nucleic acid samples and single-strand nucleic acid probes. The presently claimed invention, concerns doubled-stranded sections (>50) of nucleic acids and PNA probes. At no time, in Chen, does the probe compete with the longer complementary strand for binding with the target sequence. Indeed in nucleic acid hybridization, a short nucleic acid probe is not able to compete for hybridization against the complementary

strand. Such complex, a nucleic acid probe/nucleic acid, would melt at a temperature and other medium conditions that still favor the hybridization of the longer complementary nucleic acid strands. Further, the denaturing medium of the instantly claimed invention would render the teaching of Chen inoperative. Indeed, at no time can one observe a nucleic acid probe/nucleic acid complex, as disclosed in Chen, when the medium conditions are denaturing to the double-stranded nucleic acid sample.

In the present invention, PNA makes this possible. PNA probes remain bound to the nucleic acid sample while the medium is denaturing to double-stranded nucleic acids. There is no teaching or suggestion in Chen that PNA probes may form stable complexes with nucleic acids in medium conditions that are denaturing to long stretches of nucleic acid/nucleic acid complexes. Thus, a skilled artisan reading Rose and Chen and combining their teachings would not arrive at the presently claimed invention.

Nielsen is not seen either as curing the deficiencies of Rose, or of Rose and Chen combined. Nielsen discloses that PNA binds to duplex DNA by either displacing one strand thus forming a duplex, or by forming a triple helix. Although Nielsen discloses that recognition can take place to double-stranded DNA sequences of 5-60 base pairs, Nielsen is silent as to the effective separation of PNA/nucleic acid complexes from a medium that is denaturing to nucleic acid /nucleic acid complexes. Rather, Nielsen explicitly refers to "strand displacement recognition that occurs at physiological conditions (i.e., neutral pH, ambient (20-40°C) temperature and medium (100-150 mM) ionic strength," (see col. 9, lines 50-52). These are medium conditions under which nucleic acid hybridization occurs (and particularly for stretches of nucleic acid of greater than 50 nucleotide subunits), not denaturation as claimed in the present invention. Thus, Nielsen does not cure the deficiencies of Rose, even when combined with Chen.

With regard to claims 44 and 45, neither Rose, Chen, nor Nielsen, taken alone or in combination, teaches the use of multiple PNA sequences for multiplex detection of target sequences within a sample.

Therefore, neither Rose, Chen nor Nielsen, taken alone or in combination, teaches or suggests the invention presently claimed in claims 32, 45, and 46 and their respective depending claims. Accordingly, Applicants respectfully request that this rejection be withdrawn.

New Claims Free of the Prior Art

With regard to new independent claim 73 (see also depending claims 34, 35, 49, and 50), neither Rose, Chen, nor Nielsen, taken alone or in combination, teaches the methods of forming and detecting the PNA/nucleic acid complexes of the present invention in a denaturing medium such as urea, formamide, or salt concentrations of less than about 50 mM. Rose teaches a salt concentration of 75 mM (see page 3546, under Experimental Section). Nielsen teaches ionic strength of 100 – 150 mM (see col. 9, line 52). Both these media are favorable to nucleic acid hybridization. Chen teaches the use of urea, however, no hybridized species is observed (see page 298).

With regard to new independent claim 74 (see also claim 36), neither Rose, Chen, nor Nielsen, taken alone or in combination, teaches the methods for the detection of PNA/nucleic acid complexes while adjusting the temperature from about 95° to 65°C to a temperature of about 60° to 30°C. Rose teaches using ambient temperature, at 30°C capillary, and heating the capillary from 30° to 60°C. In the latter, detection of the complex is no longer possible.

Accordingly, Applicants respectfully submit that these new claims are free of the cited prior art.

CONCLUSION

Applicants submit that this Amendment and Reply fully addresses the rejections applied in the Office Action mailed on April 30, 1999. Accordingly, Applicants submit that the claim amendments and remarks herein place the remaining rejected claims and the new claims in condition for allowance and respectfully request early entry of such action.

Respectfully submitted,

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